Quantitative MR Measurements of Regional and Global Left Ventricular Function and Strain after Intramyocardial Transfer of VM202 into Infarcted Swine Myocardium

Short title: MR measurement of LV function after VM202 therapy

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Abstract

**Background:** Previous studies have shown beneficial effects of hepatocyte growth factor (HGF) gene on myocardial perfusion and infarction size, but not on regional strain in relation to global LV function. A non-invasive MR study was performed to determine the effect of a new HGF-gene (VM202), expressing two isoforms of HGF, on regional and global LV function.

**Methods and Results:** Pigs (8/group) were divided into; 1) controls without infarction, 2) reperfused infarcted-controls and 3) infarcted-treated (1 hour after reperfusion) with VM202 injected at eight sites. Cine, tagging and delayed enhancement MR images were acquired at 3 and 50±3 days after infarction. At 50 days, EF in infarcted-treated animals increased (38±1% to 47±2%, p<0.01) to the level of controls without infarction (52±1%, p=0.16), but decreased in infarcted-controls (41±1% to 37±1%, p<0.05). Two-dimensional strain improved in remote, peri-infarcted and infarcted myocardium. Furthermore, infarction size was smaller in infarcted-treated (7.0±0.5%) compared with infarcted-controls (13.2±1.6%, p<0.05). Histopathology showed lack of hypertrophy in myocytes in peri-infarcted and remote myocardium and formation of islands/peninsulas of myocytes in infarcted-treated, but not in infarcted-controls.

**Conclusion:** Plasmid HGF-gene caused near complete recovery of ejection fraction and improved radial and circumferential strain of remote, peri-infarcted and infarcted regions within 50 days. These beneficial effects may be explained by the combined effects of a speedy and significant infarct resorption and island/peninsulas of hypertrophied myocytes within the infarcted territory, but not by compensatory hypertrophy. The combined use of cine and tagging MR imaging provides valuable information on the efficacy of gene therapy.

**Keywords:** gene therapy, heart failure, infarction, magnetic resonance imaging, remodeling
Introduction

Accurate assessment of regional and global left ventricular (LV) function by imaging is clinically important in ischemic heart disease. Ejection fraction (EF) and end-systolic volume are important predictors of survival and are used to manage therapy. (51) Echocardiography is the most widely used technique for non-invasive imaging of LV function because of its efficacy, relatively low cost, portability and widespread availability. The limitations of 2-dimensional echocardiography include the need for adequate acoustic windows, operator dependence and the use of geometric assumptions in computing LV volumes. Gated single photon emission computed tomography (SPECT) is also used for non-invasive imaging of regional and global LV function, but it has relatively low spatial and temporal resolution. Although echocardiography and SPECT are the most popular modalities for imaging the heart, magnetic resonance (MR) imaging is more sensitive for evaluation of LV function in patients and is used as a reference method. (2, 6, 17)

Gene and stem cell therapy are under investigation as new therapies to prevent the deterioration of LV function after myocardial infarction. (36, 43) Hepatocyte growth factor (HGF) has been under investigation because of its combined angiogenic (29) and anti-fibrotic effects. (1, 47, 48) Several studies have recently shown the beneficial effects of HGF gene therapy in animal models of ischemic heart disease. (7, 19-21, 23, 24, 42) The effect of different gene therapies on EF has been shown, but the mechanism for the increase in EF has not been defined. (10, 39, 44) Investigators have attributed the increase in regional function to: 1) compensatory hypertrophy of remote myocardium, (44) and 2) viable myocytes within the infarct. (13, 39)

Left ventricular volumes, EF, regional wall thickness, systolic wall thickening (a measure of radial strain) and mass can be measured using cine MR imaging. Furthermore, tagging MR
imaging provides detailed information on circumferential and peak strain during the cardiac cycle. (11, 12, 25, 32, 33)

Recently, a new plasmid (VM202) expressing two isoforms of HGF gene became available and was tested. (39) The effects of this novel therapy on regional LV function (systolic wall thickening-radial strain and circumferential strain) and global LV function (LV volumes, ejection fraction and mass) has not previously been determined using serial MR imaging. Accordingly, we performed a non-invasive MR study to determine whether gene therapy expressing two isoforms of HGF gene could improve regional and global LV function in swine model of reperfused myocardial infarction. Histopathology was used to confirm the effects of therapy at the cellular level.

**Methods**

**Animal Preparation**

The study conformed to the Guide for the Care and Use of Laboratory Animals (NIH Publication No 85-23, revised 1996) and approval was obtained from the Institutional Committee on Animal Research. Pigs (n=24, weight 30-40 kg) were premedicated by ketamine (20mg/kg, Ketaset; Fort Dodge Labs, Fort Dodge, Iowa), xylazinie (2mg/kg, Anased; Lloyd Labs, Shenandoah, Iowa) and atropine (0.04mg/kg, Phoenix Labs, St Joseph, Missouri), anesthesized using a mixture of 1.8-2.5% isoflurane (IsoFlo; Abbott Laboratories, North Chicago, Illinois) and oxygen, and intubated as previously described. (37) The animals (n=24) were divided into three groups. I: Controls without infarction (n=8). II Infarcted-controls (n=8) and III infarcted-treated with a gene expressing two isoforms of HGF (VM202) (n=8). After thoracotomy, the pericardium was opened and the left anterior descending (LAD) was identified and occluded for 2 hours distal to
the first diagonal branch, followed by reperfusion. In group III, HGF gene (2 mg in 4 ml physiologic saline solution) was slowly injected into 4 periinfarction sites and 4 infarction sites as previously described.(39) The chest was closed layer by layer and the animals were allowed to recover for 3 days prior to the first imaging session. Four additional animals died during the LAD occlusion/reperfusion.

**HGF-gene**

VM202 (ViroMed, Co. Ltd, Seoul, Korea) was used for HGF-gene therapy. VM202 is a genomic-cDNA hybrid of human HGF gene, which expresses two isoforms of HGF, namely HGF and dHGF by way of alternative splicing. For construction of VM202, HGF-X7 was inserted into pCK DNA vector. The components of plasmid HGF gene (VM202) and their function have been described in earlier publications.(39, 41)

**MR Imaging**

A 1.5-T MR clinical scanner (Philips Medical Systems, The Netherlands) was used in all animals. Non-infarcted control animals were imaged once and infarcted animals twice, 3±0 days (acute phase) and 50±3 days (chronic phase) after the surgical procedure. In short, cine, tagging and delayed enhancement (DE) MR images were obtained of the left ventricle.(18)

**MR imaging protocols and parameters:** After initial scout imaging to obtain the short- and long-axis imaging planes, an SSFP cine sequence was used to image the whole LV in the short-axis view. Imaging parameters for the cine images were: TR/TE/flip angle = 3.5ms/1.75ms/70°, slice thickness=10mm, no slice gap, FOV=25x25 cm, matrix size=160x152, heart phases = 16. Cine images were used for assessment of LV volumes, EF, wall thickness and thickening and LV mass. A tagged turbo-field echo planar (TF-EPI) sequence, obtained in the short-axis view covering the whole left ventricle, was used. Imaging parameters for the tagged cine images were:
TR/TE/flip angle = 35ms/6.1ms/25°, slice thickness=10mm, no slice gap, FOV=24x24 cm, matrix size=128x45, heart phases = 16, EPI factor = 11, tag technique = complementary spatial modulation of magnetization (CSPAMM) with vertical and horizontal tag orientation obtained in one breath hold, line spacing = 8 mm. Delayed enhancement (DE) MRI (IR-GRE) in the short-axis view was used to locate and size the infarct so that functional assessment could be performed in infarcted, peri-infarcted and remote myocardium. DE-MRI was acquired 20min following 0.15 mmol/kg of Gd-DOTA administration (Guerbet Group, Paris, France). The imaging parameters were TR/TE/flip = 5ms/2ms/15°, slice thickness = 3mm, no slice gap, FOV=26x26cm, matrix size=256x162, the inversion time was chosen to null normal myocardium (270-325ms).

**MRI Analysis.** Global function was assessed as LV volumes, EF and LV mass by manual delineation of the endocardial and epicardial contours at end-systole and end-diastole in cine images, using the freely available software Segment v1.6(16) (http://segment.heiberg.se). LV volumes and mass were normalized to body weight in order to compare the results at the acute (32.3±0.5 kg) and chronic phase (48.4±1.4 kg).

Regional LV function was quantified in three consecutive apical, mid and basal short-axis slices through the infarcted myocardium using the centerline method. In each of these images a spoke-wheel was placed in the center of the cavity, dividing the myocardium into 8 regions starting with region 1 at the posteroseptal groove and continuing clockwise (Figure 1). Quantification of regional thickening was performed in 960 regions (8 per slice, 3 slices, 16 animals imaged twice and 8 animals imaged once). The peri-infarcted region was defined as 5 mm adjacent to the infarct in the anterior wall.
Cine MR imaging were used to determine systolic and diastolic wall thickness. Regional systolic wall thickening was determined as (end-systolic wall thickness - end-diastolic wall thickness)/end-diastolic wall thickness) *100. Tagged MR images in the same three short-axis planes as for wall thickening were used for strain analysis. Circumferential strain was calculated for all regions but only the remote, peri-infarct and infarcted regions are shown for clarity. Midwall eulerian circumferential shortening was measured using the HARP software (Diagnosoft Inc, Palo Alto, CA) (12, 32, 33) and peak circumferential strain was calculated. Peak strain values are negative during systole in normal myocardium because they express circumferential shortening. Infarct transmurality was calculated as the percentage of hyperenhanced myocardium in eight segments each for the apical, mid and basal slices used for regional function analysis by Segment v1.6(9).

**Postmortem Evaluation.** The infarcted animals were euthanized after the second imaging session (50±3 days after infarction) and the hearts were excised. The LV was sliced into 10mm short-axis slices, weighed and stained by 2% triphenyltetrazolium chloride (TTC) for confirmation of infarction. Tissue samples were obtained from the infarcted, peri-infarcted and remote normal myocardium and embedded in paraffin, sliced (5µm) and stained with hematoxylin and eosin and Masson´s trichrome. Myocyte diameters were measured at 400x magnification using a calibrated reticule in the ocular of the microscope (Olympus, Japan). The myocyte diameters were measured at the nuclear level from transversely cut cells in islands/peninsulas of myocytes within the scar tissue, peri-infarcted and remote normal myocardium.

**Statistical Analysis.** Continuous variables are presented as mean±SEM. Wilcoxon matched pairs test was used to compare LV volumes, LV ejection fraction and peak systolic strain at the
acute and chronic phase in the same group of animals. The Mann-Whitney test was used to compare LV volumes, LV ejection fraction and peak systolic strain between the groups. Two-tailed unpaired Student’s t-test was used to compare myocyte diameter and two-tailed paired Student’s t-test within the groups. A value of $p<0.05$ was considered statistically significant.

**Results**

**Global LV function on cine MR.** EF in infarcted animals at 3 days did not differ between infarcted-treated (38±1% and infarcted-control animals (41±1, $p=0.13$), (Figure 2). Both groups had significantly lower EF compared to controls without infarction (52±1%, $p<0.001$ compared to both groups). However, at 50±3 days after infarction the EF of infarcted-treated animals had increased to 47±2% ($p<0.01$ compared to 3 days and $p=0.16$ compared to controls without infarction) and decreased to 37±1% in the infarcted-controls ($p<0.05$ compared to 3 days). At 50 days, the EF of infarcted-treated animals was markedly higher than in infarcted-controls (47±2% vs. 37±1%, $p<0.001$). The increase in EF in infarcted-treated animals over time was associated with a decrease in EDV (2.15±0.10 vs. 1.73±0.10 ml/kg, $p<0.05$) and ESV (1.33±0.07 vs. 0.92±0.08 ml/kg, $p<0.01$), (Figure 2). In infarcted-controls ESV increased from 1.36±0.04 to 1.51±0.05 ml/kg ($p<0.05$) but EDV was unchanged, 2.35±0.05 vs. 2.41±0.09 ml/kg ($p=0.64$), (Figure 2). The correlation between true infarct size measured by histochemical TTC and global MR functional parameters are shown in Figure 3. The correlations show that EF decreased with increasing true infarct size ($r=0.64, y=-1.0x+50.7, P=0.007$) and that EDV ($r=0.66, y=0.069x+1.43, P=0.0055$) and ESV ($r=0.72, y=0.0615x+0.647, P=0.0017$) increased with increasing true infarct size with TTC. Normalized LV mass did not differ between the infarcted-treated and infarcted-control groups at 3 days (2.43±0.09 vs. 2.65±0.09g/kg, $p=0.08$) or 50 days
(2.16±0.08 vs. 2.32±0.10g/kg, p=0.23). Normalized LV mass in infarcted-treated animals at 50 days was not significantly different compared to controls without infarct (2.0±0.1g/kg, p=0.10), however, normalized LV mass in infarcted controls were significantly higher (p<0.05).

**Regional LV function on cine MR.** MR images at end diastolic and end-systolic phases are shown in Figures 4. Systolic wall thickening was decreased in the acute phase in infarcted and peri-infarcted regions compared to controls without infarction (Figure 5). However, there was also a trend, but not significant, towards decreased systolic wall thickening in remote myocardium compared to controls without infarction. At 50 days, systolic wall thickening in remote myocardium in infarcted-treated animals did not differ significantly compared to controls without infarction. This was in contrast to the remote myocardium of infarcted-controls, which had lower wall thickening, resulting in LV dysfunction. Wall thickness of the remote myocardium in the basal, mid and apical part was similar between the infarcted-treated (8.1±0.3, 7.5±0.3 and 6.8±0.2mm respectively) and infarcted-control groups (9.0±0.3, 8.3±0.4 and 7.0±0.6mm, respectively) 50 days after infarction (p=0.12, 0.33 and 0.87).

**Regional LV function on tagging MR.** Circumferential strain differed between the groups throughout the cardiac cycle as demonstrated in Figure 6. The peak strain of the remote, peri-infarct and infarcted regions for the basal, mid and apical slices for all animals are shown in Figure 7. At 3 days, peak strain was not significantly different between controls without infarction and infarcted-controls and infarcted-treated animals in remote myocardium. On the other hand, peak strain was significantly lower in the peri-infarcted (p<0.01) and infarcted (p<0.01) regions in both infarcted-treated and infarcted-controls compared to controls without infarction. At 50 days, peak strain was still significantly lower in peri-infarcted and infarcted regions (p<0.001) in infarcted-controls compared to controls without infarction. A small, but
significant, increase in peak strain could be seen in the infarcted regions in infarcted-controls (p<0.05). However, the increase in peak strain was more pronounced in peri-infarcted (p<0.05) and infarcted regions (p<0.001) in infarcted-treated animals. Moreover, the peak strain in the infarcted region of treated animals occurred late in the cardiac cycle compared to normal animals. Unlike infarcted-control animals, there was a significant increase (p<0.01) in peak systolic strain in basal and apical slices of remote myocardium at 50 days, in infarcted-treated animals.

**Infarct evolution.** Multislice MR images from infarcted-control and infarcted-treated animals are shown in Figure 8. The infarcts were seen as hyperenhanced regions in the LV anteroseptal wall in all animals. As previously reported, (39) the decrease in infarct size was greater in the infarcted-treated animals compared to infarcted-controls. At 50 days, the infarct decreased in both infarcted-treated (16.5±1.8% to 7.0±0.5% of LV mass, p<0.05) and infarcted-control animals (16.6±1.1% to 13.2±1.6% LV mass, p<0.05), but to a greater extent in treated animals, p<0.05. The transmurality of the infarction in the three slices used for cine and tagging analysis are shown in Figure 9. Over time transmurality of the infarction decreased in both groups, however the decrease was greater in the infarcted-treated animals. Also, an endocardial rim of viable tissue was seen in the basal part of the infarct (Figure 9).

**Histopathology**

Histochemical TTC and histological staining confirmed the formation of infarction and scar tissue in animals that underwent occlusion of the LAD. Myocardial TTC sections confirmed the lower transmurality of the infarction seen on MR-imaging in infarcted-treated animals compared to infarcted-controls (Figure 10). In infarcted-treated animals, viable myocardium was interspersed with infarcted tissue and in the basal slice of the infarction an endocardial rim of viable myocardium could be seen both on MR and TTC images. There was a high correlation
between quantification of infarct size with MR and TTC \((r=0.92, \ y=0.94x+1.3, \ P<0.001, \text{Figure 3})\).

Histopathology showed that the myocyte diameter was significantly smaller in the peri-infarcted (21±1µm) and remote myocardium (19±1µm) of infarcted-treated animals compared with infarcted-controls (26±1µm and 25±1µm respectively; \(p<0.001\) for both), (Figure 10). In infarcted-treated animals peninsulas and islands/peninsulas of viable myocytes were present within the infarct (Figure 10). The diameter of these myocytes was 29±1µm, significantly larger than peri-infarcted and remote myocardium of the infarcted-treated animals \((p<0.001\) for both).

**Discussion**

The main findings are that: 1) intramyocardially transferred plasmid HGF-gene (VM202) caused near complete recovery of EF within 50 days of infarction, 2) the therapy prevented LV remodeling associated with infarction, 3) the improvement in regional and global function in treated animals is most likely attributed to the speedy and significant resorption of infarction and island/peninsulas of hypertrophied myocytes within the infarcted territory, 4) treated animals showed no evidence of compensatory hypertrophy in remote myocardium as shown in cine MR measurement of LV mass, wall thickness or microscopic measurement of myocyte diameter compared to infarcted-control animals and 5) the complimentary use of cine and tagging MR imaging provides valuable information on the efficacy of gene therapy in ischemic heart disease, thus it can be used for non-invasive assessment of gene or cell therapy.

The importance of circumferential strain and systolic wall thickening (radial strain), using myocardial tagging and cine MR imaging, in patients with myocardial infarction or ischemic
cardiomyopathy has been recently demonstrated. (4, 14) Cine or tagging MR imaging, however, is often used in the context of an integrated MR examination, where MR sequences for imaging myocardial anatomy, perfusion, function and infarction can all be readily used and provide complimentary information. (14, 26, 35) Accordingly, in our previous study, we reported the beneficial effects of intramyocardial injection of HGF gene (VM202) on myocardial perfusion and angiogenesis. (39) The current MR study is a continuation of the previous work, where the impact of intramyocardial injection of HGF gene on the changes in regional and global LV function (remodeling indices), LV mass and natural infarct resorption were investigated. In the current study, the EF, systolic wall thickening and strain were significantly reduced in all infarcted animals (n=16) compared with controls without infarction (n=8) on the 3rd day after infarction. At 50 days, infarcted-treated animals showed marked improvement in EF, systolic wall thickening and circumferential strain compared to infarcted-controls. The improvement of EF and decline in LV volumes indicate the prevention of LV remodeling by HGF-gene therapy. Interestingly, peak systolic strain of infarcted myocardium in treated animals occurred late in the cardiac cycle, a contraction pattern associated with ischemic, but viable, myocardium. (5, 46) The improvement in wall thickening and strain seen in treated animals can be attributed to: 1) speedy infarct resorption in treated animals resulting in a non-transmural infarction and 2) the presence of viable myocyte islands/peninsulas within the scar tissue. The improvement in LV function, however, was not linked to compensatory hyperfunction or hypertrophy in remote myocardium as shown by the lack of increase in LV mass, wall thickness or cellular diameters on histology. These findings differs from previous findings using intra-coronary injection of adenoviral gene transferred FGF-5 to hibernating myocardium, (28, 44) where the investigators observed massive
increase in LV mass at 2 weeks (29% increase) and 4 weeks (55% increase) after intracoronary FGF-therapy.

Previously, we have shown the beneficial effects of intramyocardial injection of adeno-associated viral vector-encoding vascular endothelial growth factor (AAV-VEGF) gene into peri-infarcted and infarcted myocardium, using the same animal model, surgical procedure and MR imaging protocol. (18, 40) Intramyocardial injection of AAV-VEGF gene into swine myocardium decreased infarction size, increased regional perfusion and increased regional function in treated animal compared to infarcted-controls. However, AAV-VEFG gene did not increase the global function EF over time or cause the formation of viable islands/peninsulas in the infarcted myocardium. Furthermore, in a canine study, Ferrarini et al. used AAV-VEGF gene and found that intramyocardially injected gene improved regional function but not global function and that the therapy enhanced the formation of viable myocardium in the infarcted region.(10) Similar findings were observed in sheep.(50) Unlike AAV-VEGF gene therapy (10, 18, 40), HGF-gene in the present MR study showed a substantial improvement in both regional and global function over time and prevented the LV remodeling associated with myocardial infarction.(8, 34) Therefore, the effects of HGF-gene therapy on restoration of LV function is more pronounced compared to VEGF-gene therapy, and in this respect our findings support the notion of using HGF gene in the future design of clinical studies.(29)

In the current study, the HGF gene caused significant reduction in infarct size and transmurality. In a clinical MR study, Kim et al. found that the transmural extent of infarction is predictive of post-revascularization functional recovery independent of LV function.(22) They also found a close correlation between the transmural extent of infarction and a lower likelihood of functional recovery.
Unlike FGF or VEGF, HGF is characterized by its anti-fibrotic effect. The anti-fibrotic mechanism of HGF has been shown to be mediated through 1) inhibition of synthesis of extracellular matrix via the inhibition of TGF-β expression and 2) stimulation of degradation of extracellular matrix through activation of MMP-1 and uPA. Previous studies have shown that HGF acts on a molecular level by reducing the production of oxygen free radicals during ischemia and reperfusion, which could trigger necrosis and enhances the expression of Bcl-2 and Bcl-XL, known inhibitors of apoptosis. These anti-fibrotic and anti-apoptotic effects of HGF-gene treatment may explain the greater decrease in infarct transmurality in the infarcted-treated group compared to infarcted-controls in our study.

The angiogenic properties of HGF have been shown to be explained by a mitogenic action as well as an accelerated regeneration of the endothelial cells. Morishita et al. found that HGF stimulates the migration of vascular smooth muscle cells (VSMC) to the sprouting new vessels. The effect on VSMC may explain why HGF, as opposed to VEGF, does not increase vascular permeability and as a consequence does not cause oedema formation. A recent study showed for the first time that post-ischemic myocardial dysfunction (stunning) results from myofibrillar edema.

The mechanisms of formation of islands/peninsulas of viable myocytes in the peri-infarcted region may be explained by findings in a recent rat study by Gonzales et al. They found that administration of IGF-1 and HGF modified cardiac progenitor cells behaviour. The study showed myocardial regeneration from cardiac progenitor cells forming myocytes, coronary arterioles and capillaries. Interestingly, the investigators stated that “clusters of regenerated myocytes replaced foci of myocardial damage”. Thus, the presence of viable myocytes at the
peri-infarcted region can be explained by HGF rejuvenating cardiac progenitor cells to form new viable myocytes.

In the present study, intramyocardial injection of the HGF-gene were performed at eight sites to cover most of the ischemic territory to ensure fair distribution of the therapy. Furthermore, the size of VM202 is relatively low (7376 base pairs) and the gene is therefore expected to distribute by diffusion transport in the tissue. Intramyocardial delivery approach in open chest model has been previously used.(13) We recognized that the inferoseptal wall can not be reached using open chest models, therefore we recently developed a percutaneous transendocardial route for gene delivery. (38)

Limitations of the present study were lack of 1) measurement of gene expression (HGF protein), 2) placebo injections and 3) non-invasive visualization of new collateral vessels. The gene expression was not the scope of the present MR study. It should be noted, however, that the expression of HGF protein in plasma and tissue has been well documented in several earlier studies.(1, 7, 27) and that individual levels of angiogeneic protein are quite variable.(45)

In conclusion, intramyocardially transferred plasmid HGF-gene caused almost complete recovery of ejection fraction within 50 days of infarction, prevented LV remodeling and increased radial (systolic wall thickening) and circumferential strain of remote, peri-infarcted and infarcted regions. This may be explained by the combined effects of a speedy and significant infarct resorption and island/peninsulas of hypertrophied myocytes within the infarcted territory, but not by compensatory hypertrophy. The combined use of cine and tagging MR imaging provides valuable information on the efficacy of gene therapy.
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References:


Figure Legends:

**Figure 1.** MR-images 3 days after infarction. Three short axis levels through the infarct, basal (top row), mid (mid row) and apical (bottom row), were used for analysis of strain and systolic wall thickening. Left panels: DE-MRI short-axis images showing the anteroseptal infarct as a bright region. The spoke wheel and the numbers 1 to 8 in the top left image shows the eight sectors used for measurement of systolic wall thickening (Figure 3). White arrows indicate the position of the three measurements points used for strain analysis: A remote, B peri-infarct, and C infarct. Middle and right panels show the corresponding MR-tagging images used for strain analysis in end-diastole (ED) and end-systole (ES). The outer and inner circles indicate the delineation of the epicardium and endocardium respectively. The middle circles show where circumferential strain was measured.

**Figure 2.** Global functional parameters. A, Ejection fraction (EF), B, End diastolic volume normalized to body weight (EDV/BW) and C, end systolic volume normalized to body weight (ESV/BW) for controls without infarction (No inf, grey bars) infarcted-controls (Inf.C) and infarcted-treated (Inf.T) animals at 3 days (black bars) and 50 days (white bars). ** p<0.01 and *** p<0.001 when comparing infarcted-controls and infarcted-treated animals. § p<0.05, §§ and p<0.01 when comparing the functional parameters at 50 and 3 days, within the groups. † p<0.05, †† p<0.01 and ††† p<0.001 when comparing functional parameters in the infarcted groups with the control animals without infarction.

**Figure 3.** Correlation between MR parameters and infarction size measured by histochemical TTC for infarcted-treated and infarcted-control animals. The correlation show that ejection fraction (A) decreases and LV volumes (B, C) increases with increasing infarct size. In addition, there was a high correlation between infarct size quantified with MR and TTC (D).
Figure 4. Cine MR-imaging showing the evolution of regional LV function. At 3 days there is a decreased wall thickening in the anteroseptal wall in both animals. However, at 50 days the images differ with dilatation of the ventricle and thinning of the anteroseptal wall in the infarcted-control animal, resulting in decreased regional function. In the infarcted-treated animal, there is not as prominent dilatation of the ventricle or thinning of the anteroseptal wall. Furthermore, the function of remote myocardium in the lateral wall is higher in the infarcted-treated animal compared to infarcted-control. ED end-diastole, ES end-systole.

Figure 5. Regional systolic wall thickening, a measure of radial strain (mean±SEM) for controls without infarction (grey bars), infarcted-controls (black bars) and infarcted-treated animals (white bars) at 3 days (left panels) and 50 days (right panels). * p<0.05, ** p<0.01 when comparing systolic wall thickening at 3 and 50 days. § p<0.05, §§ p<0.01 and §§§ p<0.001 when comparing systolic wall thickening in infarcted-treated animals to infarcted-controls. † p<0.05, † p<0.01 and † p<0.001 when comparing systolic wall thickening in infarcted animals with controls without infarction.

Figure 6. Circumferential eulerian strain (Ecc) throughout the RR-interval at 3 days (left panels) and 50 days (right panels) is shown for infarcted-controls (open squares) and infarcted-treated animals (solid circles). Strain values shown are mean±SEM for all animals. Circumferential strain for control animals without infarction (crosses and dotted lines) is shown for comparison. * p<0.05, ** p<0.01 and *** p<0.001, comparing peak strain in infarcted-treated animals to infarcted-controls. † p<0.05 and †† p<0.01 comparing peak strain at 3 and 50 days within the groups.

Figure 7. Peak circumferential strain in control animals without infarction (grey bars), infarcted-controls (black bars) and infarcted-treated animals (white bars) at 3 days (left panels) and 50
days (right panels) for the basal, mid and apical short-axis MR-images. * p<0.05, ** p<0.01 and *** p<0.001 when comparing peak strain in infarcted-treated animals to infarcted-controls. § p<0.05, §§ p<0.01 and §§§ p<0.001 when comparing peak strain at 3 and 50 days within the groups. † p<0.05, † p<0.01 and † p<0.001 when comparing peak circumferential strain in infarcted animals with controls without infarction.

**Figure 8.** DE-MR-images showing the infarct as a hyperenhanced region in the anteroseptal wall (white arrows denote the limits of the infarct). Over time the infarct becomes thinner and some infarct resorption can be seen. However, infarct resorption is greater in the infarcted-treated animal.

**Figure 9. A.** Infarct transmurality (mean±SEM) measured by MRI for infarcted-controls (left panels) and infarcted-treated animals (right panels) at 3 days (black bars) and 50 days (white bars). The anatomic positions of the circumferential myocardial segments are shown in Fig.1. * p<0.05 when comparing infarct transmurality between 3 and 50 days. **B.** TTC images illustrating the difference in infarct transmurality between infarcted-control (left) and infarcted-treated (right) animals. Black arrowheads indicate the transmural extent of the infarct in the infarcted-control. Note the difference in wall thickness between animals in the infarcted region, and the endocardial rim of viable myocardium (white arrowheads) in the infarcted-treated animal.

**Figure 10. A.** Histopathological findings in the infarcted and peri-infarcted regions at 50 days. Left panels show infarcted-controls and right panels infarcted-treated animals. Top row: Low resolution of Masson’s trichrome staining showing the border between infarct and peri-infarcted myocardium. In infarct-controls the border is smooth with a few thick-walled arterioles and few myocytes within the infarct. This is in contrast with the irregular border between infarct and peri-
infarcted myocardium with an abundance of thin walled arterioles, in addition to a few thick walled arterioles in infarct-treated animals. Also, island/peninsulas of myocytes can be seen within the infarct in infarct-treated animals. Calibration bars in bottom right corner equals 200 µm. Bottom row: High resolution of Masson trichrome stain. The smooth border of the infarct in the infarcted-controls is seen in contrast to the island/peninsula of hypertrophied myocytes within the scar tissue in the infarcted-treated animal. Calibration bars in bottom right corner equals 20 µm. **B.** Histopathologic measurements of myocyte dimensions in infarcted-controls (black bars) and infarcted-treated animals (white bars). *** p<0.001, §§§ p<0.001 compared to myocyte dimensions in infarcted region.
A

\[ r = 0.64 \]
\[ y = -1.0x + 50.7 \]

B

\[ r = 0.66 \]
\[ y = 0.069x + 1.43 \]

C

\[ r = 0.72 \]
\[ y = 0.0615x + 0.647 \]

D

\[ r = 0.92 \]
\[ y = 0.94x + 1.3 \]
Myocyte diameter (μm)

- Infarcted-control
- Infarcted-treated

**A**

Comparison of tissue sections labeled "Infarcted-control" and "Infarcted-treated".

**B**

Bar chart showing myocyte diameters for different regions:
- Infarct
- Peri-infarct
- Remote

Statistical significance indicated by asterisks:
- ***: high significance
- $$$: moderate significance

Links to images and data for detailed analysis.